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PERSPECTIVE

Antioxidant therapy in male infertility: fact or fiction?

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Infertile men have higher levels of semen reactive oxygen species (ROS) than do fertile men. High levels of semen ROS can cause sperm dysfunction, sperm DNA damage and reduced male reproductive potential. This observation has led clinicians to treat infertile men with antioxidant supplements. The purpose of this article is to discuss the rationale for antioxidant therapy in infertile men and to evaluate the data on the efficacy of dietary and *in vitro* antioxidant preparations on sperm function and DNA damage. To date, most clinical studies suggest that dietary antioxidant supplements are beneficial in terms of improving sperm function and DNA integrity. However, the exact mechanism of action of dietary antioxidants and the optimal dietary supplement have not been established. Moreover, most of the clinical studies are small and few have evaluated pregnancy rates. A beneficial effect of *in vitro* antioxidant supplements in protecting spermatozoa from exogenous oxidants has been demonstrated in most studies; however, the effect of these antioxidants in protecting sperm from endogenous ROS, gentle sperm processing and cryopreservation has not been established conclusively.
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RELATIONSHIP BETWEEN OXIDATIVE STRESS AND SPERM DYSFUNCTION

Seminal oxidative stress (OS) results from an imbalance between reactive oxygen species (ROS) production and ROS scavenging by seminal antioxidants. Seminal OS is believed to be one of the main factors in the pathogenesis of sperm dysfunction and sperm DNA damage in male infertility.[1–4] Indeed, it is estimated that 25% of infertile men possess high levels of semen ROS, whereas fertile men do not have high levels of semen ROS.[1,4–6] Although a controlled production of these ROS is required for sperm physiology (sperm hyperactivation, capacitation and acrosome reaction) and for natural fertilization,[7–9] the excessive production of ROS by immature germ cells and leukocytes causes sperm dysfunction (lipid peroxidation, loss of motility and sperm DNA damage).[9,10]

Spermatozoa are particularly susceptible to oxidative injury due to the abundance of plasma membrane polyunsaturated fatty acids.[10–12] These unsaturated fatty acids provide fluidity that is necessary for membrane fusion events (e.g., the acrosome reaction and sperm–egg interaction) and for sperm motility. However, the unsaturated nature of these molecules predisposes them to free radical attack and ongoing lipid peroxidation throughout the sperm plasma membrane. Once this process has been initiated, accumulation of lipid peroxides occurs on the sperm surface (this results in loss of sperm motility) and oxidative damage to DNA can ensue.[13,14]

SEMINAL ANTIOXIDANT CAPACITY AND SPERM DYSFUNCTION

Seminal plasma and spermatozoa themselves are well endowed with an array of protective antioxidants to protect spermatozoa from OS, especially, at the post-testicular level.[6,15,16] Seminal plasma contains a number of high-molecular weight enzymatic antioxidants (superoxide dismutase, catalase and glutathione peroxidase) and a deficiency in these enzymes has been reported to cause sperm DNA damage and male infertility.[1,7,10,17–19] Seminal fluid also contains non-enzymatic antioxidants (ascorbic acid, α-tocopherol, pyruvate, glutathione, *L*-carnitine, taurine and hypotaurine)[20–23] which constitute the bulk of seminal antioxidant capacity. In addition, urate,[24] pyruvate,[11,25] albumin, beta carotenes and ubiquinol[26] have been detected in seminal plasma.

A number of investigators have shown that seminal antioxidant capacity is suppressed in infertile men with high ROS levels compared to men with normal levels of ROS.[20,27,28] However, it is unclear whether reduced semen antioxidant capacity necessarily causes sperm dysfunction (including sperm DNA damage).[1,3,29,30] Indeed, there is some controversy as to whether the high ROS levels detected in the semen of infertile men are due to increased ROS production, decreased ROS scavenging capacity or both.[21,31] If the high semen ROS levels are due (at least in part) to a decreased ROS scavenging capacity of semen, it would support the use of dietary antioxidant supplementation.[21,31]

Although a relationship between male infertility and systemic antioxidant deficiency has not been reported to date, it is possible that a subset of infertile men may be at risk for antioxidant deficiency, particularly, vitamin C deficiency.[32] We suspect that infertile men with specific lifestyles (e.g., smoking, increased alcohol intake and dieting) may be at high risk for antioxidant or vitamin deficiency, but this remains to be tested.[33,34] Recently, investigators evaluated dietary antioxidant intake (vitamins C, E or β-carotene) and sperm DNA damage in a cohort of fertile men, but failed to identify any relationships between these parameters.[35]

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TREATMENT OF OXIDATIVE STRESS

Treatment of oxidative stress should first involve strategies to reduce or eliminate stress-provoking conditions including smoking, varicocele, genital infection, gonadotoxins and hyperthermia. The rationale for treating infertile men with oral antoxidants is based on the premise that seminal oxidative stress (common in infertile men) is due in part to a deficiency in seminal antioxidants. The practice of prescribing oral antioxidant is supported by the lack of serious side effects related to antioxidant therapy, although few studies have carefully evaluated the risk of overtreatment with antioxidants.[36] Ideally, an oral antioxidant should reach high concentrations in the reproductive tract and replete a deficiency of vital elements important for spermatogenesis. Additionally, the antioxidant supplement should augment the scavenging capacity of seminal plasma and reduce the levels of semen ROS.[1] However, the levels of semen ROS should not be entirely suppressed (by oral antioxidants) as this may impair normal sperm functions (e.g., sperm capacitation and hyperactivation) that normally require low levels of ROS.[7,9,19]

To date, over 100 clinical and experimental studies have examined the effect of antioxidants on sperm parameters. Despite this large body of literature, it is not possible to establish firm conclusions regarding the optimal antioxidant treatment for infertile men because the published studies report on different types and doses of antioxidants, the studies are small, the end points vary and few of the studies are placebo-controlled.[1,6,15] Moreover, the presumed mechanism of action of antioxidants in the treatment of male infertility (i.e., suppression of seminal OS) has not been confirmed because few studies have evaluated seminal OS and/or antioxidant capacity before and after treatment.[37,38]

Effect of oral (dietary) antioxidants on sperm dysfunction and DNA damage

While there is a good body of literature on the effect of oral antioxidants on sperm parameters (including sperm DNA integrity), no study has established the optimal dose, duration of treatment or subpopulation of infertile patients who might benefit most from antioxidant therapy (isolated asthenozoospermia, oligoasthenoteratozoospermia, sperm DNA damage or all). Many small, uncontrolled studies have shown a significant improvement in semen parameters following different doses and types of antioxidant therapy.[6,15] The most commonly studied oral antioxidants (or antioxidant enzyme cofactors) include vitamin C, vitamin E, selenium, zinc, glutathione, *L*-carnitine and *N*-acetyl cysteine.

The randomized controlled trials (RCTs) on antioxidant therapy for male infertility generally demonstrate that treatment with antioxidants has a beneficial effect (in terms of semen parameter improvements), whereas no significant effect is seen in the placebo group[37,39–61] (**Tables 1 and 2**). The variable treatment outcomes in different studies could be due to differences in vitamin dosages, duration of treatment and patient population.[6,15]

One RCT evaluated the effects of vitamin C alone and reported a significant improvement in sperm parameters in the treatment arm only.[46] Six RCTs evaluated the effects of vitamin E alone or in combination with vitamin C or selenium. Two of these studies reported a significant improvement in sperm motility[39,41] and one reported a significant improvement in sperm DNA integrity [59] in the treatment arm only. In contrast, three RCTs reported no significant improvement in sperm parameters after vitamin E±C treatment,[56–58] although sperm–zona binding improved in one of these studies.[56] Five RCTs evaluated the effects of zinc alone or in combination with folic acid and all five reported a significant improvement in sperm parameters in the treatment arm only.[47,50–55] Three RCTs evaluated the effects of selenium alone or in combination with *N*-acetyl cysteine and two of the three studies reported a significant improvement in sperm parameters in the treatment arm only.[43,48,54] Four RCTs evaluated the effects of *L*-carnitine alone or in combination with *L*-acetyl carnitine and three of the four reported a significant improvement in sperm parameters in the treatment arm only.[42,44,49,60] Three RCTs evaluated the effects of *N*-acetyl cysteine alone or in combination with selenium and all three reported a significant improvement in sperm parameters in the treatment arm only.[45,54,61]

Several investigators have examined the effect of antioxidant therapy on sperm DNA integrity because sperm DNA damage may be caused, at least in part, by oxidative stress.[15,22,29,53,62–69] In addition, sperm DNA damage is a more reliable outcome measure than sperm concentration or motility because measures of sperm DNA damage exhibit a lower degree of biological variability than conventional semen parameters.[70–72] Treatment with oral antioxidants has generally been associated with improvement in sperm DNA integrity and in some cases pregnancy rates after assisted reproduction, although most of these studies are small and few are randomized placebo-controlled trials (**Table 3**).[1] To date, none of the studies on sperm DNA damage and oral antioxidants have estimated seminal oxidative stress, seminal vitamin levels or used oxidative DNA damage (e.g., by estimation of 8-hydroxy-2′-deoxyguanosine (8-OHdG)) as a selection criterion for monitoring the response to antioxidant treatment.[1,2,73] As such, the precise mechanism of action of these antioxidant supplements on sperm DNA quality is unknown.

Effect of *in vitro* antioxidants on sperm dysfunction and DNA damage

The generation of oxidative stress in the *in vitro* environment, either by direct application of ROS (exogenous) or activation of intrinsic sperm ROS (endogenous), has been associated with clinical evidence of lipid peroxidation, sperm dysfunction and sperm DNA damage.[13,14,74–78] This is particularly important in the context of *in vitro* fertilization where seminal plasma is removed during semen processing and the toxic oxygen metabolites (generated by immature spermatozoa and leukocytes) are able to attack spermatozoa without being protected by seminal plasma antioxidants. In addition, the detrimental effect of oxidative stress on sperm functional competence can be exaggerated by the *in vitro* sperm processing techniques (centrifugation and prolonged incubation) that usually precede assisted reproductive techniques.[1,14,75,79]

ROLE OF *IN VITRO* ANTIOXIDANTS IN PROTECTING SPERMATOZOA FROM EXOGENOUS ROS

Attenuating the effects of exogenous ROS is clinically relevant as many of the semen samples from infertile men contain abnormal spermatozoa and leukocytes, and, these cells have the potential to generate exogenous ROS.[76] Antioxidants such as vitamin E, catalase and glutathione have been shown to protect sperm motility from the effects of exogenous ROS (**Table 4**).[11,80] In contrast, superoxide dismutase is less effective in preventing the loss of motility due to exogenous oxidants.[11,80] Altogether, these data suggest that hydrogen peroxide (H_2O_2) is the most sperm-toxic ROS. Antioxidants have also been shown to protect the sperm

Table 1 Summary of studies (RCTs) with positive effect of oral antioxidants on sperm parameters

Study	*Antioxidant and dose*	*Duration of treatment*	*Study population*	*Sample size (n)*	*Improvement*
Comhaire *et al.* (2005)[37]	Astaxanthin 16 mg	3 months	Unexplained infertility	Treated 11 Control 19	Motility Concentration
Suleiman *et al.* (1996)[39]	Vitamin E 300 mg	6 months	Asthenospermia	Treated 52 Control 35	MDA Motility
Lenzi *et al.* (1993)[40]	Glutathione 600 mg alternate days	2 months	Infertility with varicocele or genital tract infection	Treated 10 Control 10 Crossover	Motility Morphology
Keskes-Ammar *et al.* (2003)[41]	Vitamin E 400 mg and selenium 225 mg	3 months	Infertility	Treated 28 Control 20	MDA Motility Concentration
Balercia *et al.* (2005)[42]	LC 3 g d^{-1}, LAC 3 g d^{-1}, a combination of LC 2 g d^{-1} and LAC 1 g d^{-1}	6 months	Asthenospermia	Treated 44 Control 15	Motility
Scott *et al.* (1998)[43]	Selenium 100 mg or/with vitamin A 1 mg, vitamin C 10 mg and vitamin E 15 mg	3 months	OAT, subfertile	Treated 46 Control 18	Motility
Cavallini *et al.* (2004)[44]	LC 2 g d^{-1}±LAC 1 g d^{-1} ±cinnoxicam 1×30 mg	6 months	Idiopathic OAT Varicocele associated OAT	Treated 118 Control 207	Concentration Motility Morphology (except in high-grade varicocele)
Ciftci *et al.* (2009)[45]	NAC 600 mg	3 months	Idiopathic infertility	Treated 60 Control 60	Motility Viscosity Volume
Dawson *et al.* (1992)[46]	Vitamin C 1 g d^{-1} or 200 mg d^{-1}	1 month	Heavy smokers	Treated 50 Control 25	Sperm quality Sperm parameters
Ebisch *et al.* (2006)[47]	Folic acid 5 mg Zinc 66 mg	26 weeks	Subfertile	Treated 47 Control 40	Concentration
Lenzi *et al.* (2003)[49]	LC 2 mg	6 months	OAT	Treated 43 Control 43 Crossover	Concentration, motility
Mahajan *et al.* (1982)[50]	Zinc 50 mg	6 months	Gonadal dysfunction in uremic patients	Treated 10 Control 10	Concentration
Omu *et al.* (2008)[51]	Zinc 400 mg±vitamins E 20 mg and C 5 mg	3 months	Asthenospermia	Treated 37 Control 8	Mainly motility Concentration, morphology
Omu *et al.* (1998)[52]	Zinc 500 mg	3 months	Asthenospermia	Treated 49 Control 48	Concentration Motility
Piomboni *et al.* (2008)[53]	Beta-glucan 20 mg, papaya 50 mg, lactoferrin 97 mg, and vitamin C 30 mg and vitamin E 5 mg	3 months	Asthenoteratozoospermia	Treated 36 Control 15	Motility Morphology
Safarinejad and Safarinejad (2009)[54]	Selenium 200 mg±NAC 600 mg	26 weeks	Asthenospermia	Treated 468 Control 118	Motility Concentration Morphology
Wong *et al.* (2002)[55]	Folic acid 5 mg Zinc 66 mg	26 weeks	Subfertile men	Treated 94 Control 99	Concentration
Paradiso Galatioto *et al.* (2008)[61]	NAC 600 mg+ vitamins− minerals		Persistent oligospermia	Treated 20 Control 22	Concentration

Abbreviations: LC, *L*-carnitine; LAC, *L*-acetyl carnitine; MDA, malondialdehyde; NAC, *N*-acetyl cysteine; OAT, oligoasthenoteratospermia; RCT, randomized controlled trial.

DNA from the effects of exogenous ROS (**Table 4**).[81–84] This is highly relevant as sperm DNA damage may impact on reproductive outcomes after assisted reproductive technologies.[6] Indeed, sperm DNA damage has been associated with reduced pregnancy rates with intrauterine insemination, and, to a lesser extent with conventional *in vitro* fertilization.[5,85,86]

ROLE OF *IN VITRO* ANTIOXIDANTS IN PROTECTING SPERMATOZOA FROM ENDOGENOUS ROS

Spermatozoa can be stimulated to generate ROS using a variety of agents (e.g., NADPH and estrogens) and this ROS production can potentially impair sperm function.[87] In contrast to the beneficial effect of antioxidants in protecting spermatozoa from exogenous ROS, antioxidants appear to be of limited value in protecting spermatozoa from endogenous ROS production.[14] Twigg *et al.* demonstrated that SOD, catalase or both are ineffective, whereas albumin is effective in protecting spermatozoa from loss of motility due to endogenous ROS generation.[14] These studies stress the importance of using gentle semen processing protocols (e.g., low centrifugation force) so as to minimize the production and adverse impact of endogenous ROS.

Similarly, antioxidants appear to be of limited value in protecting the DNA of normal spermatozoa (with normal chromatin compaction)

Table 2 Summary of studies (RCTs) with no effect of oral antioxidants on sperm parameters

Study	*Antioxidant and dose*	*Duration of treatment*	*Study population*	*Sample size (n)*	*No improvement*
Hawkes *et al.* (2009)[48]	Selenium 300 mg d^{-1}	48 weeks	Normozoospermia	Treated 20 Control 22	Motility Morphology
Kessopoulou *et al.* (1995)[56]	Vitamin E 600 mg	3 months	Infertility with high ROS	Crossover Treated and control 30	Concentration, motility Morphology
Moilanen *et al.* (1993)[57]	Vitamin E 100 mg	3 months	Unexplained infertility IUI	Treated 6 Control 9	Concentration Motility Morphology
Rolf *et al.* (1999)[58]	Vitamin C 1000 mg, vitamin E 800 mg	56 days	Asthenospermia	Treated 15 Control 16	Concentration Motility Morphology Viability
Greco *et al.* (2005)[59]	Vitamins C and E, 1 g d^{-1}	2 months	Idiopathic infertility	Treated 32 Control 32	Concentration Motility Morphology
Sigman *et al.* (2006)[60]	Carnitine 1000 mg, *L*-acetyl carnitine 500 mg	24 weeks	Asthenospermia	Treated 12 Control 9	Motility

Abbreviations: IUI, intrauterine insemination; RCT, randomized controlled trial; ROS, reactive oxygen species.

Table 3 Effect of dietary antioxidant supplements on sperm DNA integrity

Study	*Patients/test*	*Treatment(s)*	*Sample size (n)*	*Results*
Infertile men with high sperm DNA fragmentation levels or oxidative stress				
Greco *et al.* (2005)[59]	Infertility	Vits C 1 g, E 1 g	32	Rx (2 months): ↓DD (22%→9%)
	TUNEL >15%		32	Placebo group: no effect on DD (22%–22%)
Gil-Villa *et al.* (2009)[62]	Pregnancy loss	Vits C, E zinc, β-carotene	9	Rx (3 months): 6 (of 9) couples got pregnancy
	↑LPO or DFI			No control group
Greco *et al.* (2005)[63]	1 failed ICSI	Vits C 1 g, E 1 g	38	Rx (2 months): ↓DD in 76%, 48% ICSI pregnancy
	TUNEL >15%			No control group
Menezo *et al.* (2007)[66]	2 failed ICSI	Vits C, E (400 mg), zinc, Se,	57	Rx (90 days): ↓sperm %DFI (32%→26%: by 19%),
	DFI >15%	β-carotene		but ↑sperm %HDS (17.5%–25.5%: by 23%)
	Decond >15%			No control group
Tremellen *et al.* (2007)[67]	Male infertility	Menevit (lycopene, vits C,	36	Rx (3 months): 39% ICSI pregnancy rate, but no ↑ in embryo quality, no post-Rx DD
	TUNEL >25%	E, zinc, Se, folate, garlic)	16	Placebo group: 16% ICSI pregnancy rate
Tunc *et al.* (2009)[68]	Male infertility	Menevit (lycopene, vits C,	45	Rx (3 months): ↓DD (22%→18%), ↓ROS production and ↑sperm protamination
	↑Semen OS	E, zinc, Se, folate, garlic)		No control group
Unselected infertile men				
Piomboni *et al.* (2008)[53]	Asthenospermia	Vits C, E, β-glucan, papaya,	36	Rx (90 days): ↑motility and morph but not DD
	AO stain	lactoferrin	15	Control group: no effect
Kodama *et al.* (1997)[65]	Male infertility	Vits C, E (200 mg),	14	Rx (2 months): ↓ in 8-OHdG (1.5→1.1/10^5 dG)
	8-OHdG	glutathione (400 mg)	7	Control group: no change in 8-OHdG levels

Abbreviations: 8-OHdG, 8-hydroxy-2′-deoxyguanosine; AO, acridine orange; DD, DNA damage; Decond, decondensation; DFI, DNA fragmentation index; LPO, lipid peroxidation; OS, oxidative stress; Rx, treatment; ROS, reactive oxygen species; Se, selenium; TUNEL, terminal nucleotidyl transferase-mediated dUTP nick end labeling; vit, vitamin.

Table 4 Role of *in vitro* antioxidants in protecting spermatozoa from the loss of motility and DNA damage due to exogenous ROS

Study	*Exogenous ROS*	*Antioxidant supplement and results*
Sperm motility		
de Lamirande and Gagnon (1992)[11]	X+XO	Catalase protects sperm from X+XO-induced loss of motility
		SOD, DTT or GSH less effective in protecting sperm motility from ROS
Griveau and Le Lannou (1994)[93]	X+XO	Catalase protects sperm from X+XO-induced loss of motility
		SOD or mannitol ineffective in protecting sperm motility from ROS
Sperm DNA		
Lopes *et al.* (1998)[81]	X+XO	GSH+hypotaurine protect sperm from X+XO-induced DD
		Catalase protects sperm from X+XO-induced DD
		N-acetylcysteine protects sperm from X+XO-induced DD
Potts *et al.* (2000)[82]	H_2O_2+Fe+ADP	Seminal plasma (>60% v/v) lowers oxidative sperm damage (↓DD, LPO)
Russo *et al.* (2006)[83]	H_2O_2	Propolis lowers oxidative sperm damage (↓LPO, DD, LDH)
	Benzopyrene	(Propolis—a natural resinous hive product)
	H_2O_2+Fe+ADP	
Sierens *et al.* (2002)[84]	H_2O_2	Isoflavones, vitamins C and E protect sperm from H_2O_2-induced DD
		(isoflavones: genistein, equol). Dose effect noted.

Abbreviations: ADP, adenosine diphosphate; DD, DNA damage; GSH, glutathione; LDH, lactate dehydrogenase; LPO, lipid peroxidation; X, xanthine; XO, xanthine oxidase.

from endogenous ROS production (e.g., NADPH-induced or centrifugation-induced).[14,77,88,89] In samples with poor morphology and poor sperm chromatin compaction, antioxidants may protect the sperm DNA from endogenous ROS production, as these samples are more vulnerable to oxidative stress.[90,91] In support of these clinical observations, experimental (animal) studies suggest that the spermatozoa of infertile men may be more susceptible to oxidative injury *in vitro* but benefit more so from antioxidants than the spermatozoa of fertile men.[92]

ROLE OF *IN VITRO* ANTIOXIDANTS IN PROTECTING SPERMATOZOA FROM SEMEN PROCESSING

Several studies have reported on the effects of antioxidants in preventing the decline in sperm motility after semen processing and incubation (**Table 5**). These studies have clinical relevance because it is important to maximize sperm motility prior to assisted reproductive techniques such as intrauterine insemination and standard *in vitro* fertilization. The available studies report conflicting results regarding the effects of antioxidants in preventing the loss of sperm motility during sperm processing such as centrifugation and incubation. Some studies have shown that antioxidants (e.g., vitamin E, glutathione, *N*-acetyl cysteine, catalase and ferulic acid) are effective in reducing ROS levels and in preventing the decline in sperm motility during sperm processing.[93–96] In contrast, other studies have reported that antioxidants (e.g., glutathione and catalase) are ineffective in protecting spermatozoa from the loss of motility during sperm processing.[97–99] It is important to note that sperm samples from infertile men may be more susceptible to oxidative injury (from semen processing) and be afforded greater protection by antioxidants than samples from fertile men.[92]

Antioxidants appear to be of limited value in protecting sperm DNA from gentle semen processing (e.g., incubation or density-gradient centrifugation) (**Table 5**).[98–101] In some cases, antioxidants supplementation *in vitro* (e.g., combination of vitamins C and E) may cause sperm DNA damage.[99,101]

ROLE OF *IN VITRO* ANTIOXIDANTS IN PROTECTING SPERMATOZOA FROM CRYOPRESERVATION AND THAWING

Several studies have evaluated the role of antioxidants in protecting spermatozoa from the loss of motility that occurs following cryopreservation and thawing. Most studies have reported on the use of pentoxifilline (an antioxidant and phosphodiesterase inhibitor). Some studies have shown that pentoxifilline improves post-thaw sperm motility and/or sperm function,[102–105] whereas others have demonstrated that this antioxidant does not have a beneficial effect.[106] Other antioxidants (vitamins E and C and rebamipide) have been used to enhance post-thaw motility; however, the results have been modest.[107,108] Several studies have also examined the role of antioxidants in protecting sperm DNA from injury following cryopreservation and thawing. Most studies have shown that antioxidants (vitamin C, catalase, resveratrol and genistein) can protect the sperm DNA from oxidative injury during cryopreservation and subsequent thawing[109–112] (**Table 6**). In contrast, Taylor *et al.* reported that the antioxidant vitamin E does not protect sperm DNA during cryopreservation.[113]

Taken together, the data suggest that antioxidants are generally effective in protecting spermatozoa from the effects of cryopreservation and thawing. However, the technique of cryopreservation and

Table 5 The effect of *in vitro* antioxidants on sperm motility and DNA integrity during semen processing

Study	*Parameter*	*Semen processing*	*Antioxidant supplement and results*
Motility			
Griveau and Le Lannou (1994)[93]	Motility	CF at 400 *g*×2 Swim-up	DTT, catalase, SOD or GSH improve motility
Oeda *et al.* (1997)[94]	Motility ROS	2 h incubation	NAC lowers semen ROS levels NAC improves sperm motility
Verma and Kanwar (1999)[95]	Motility LPO	6 h incubation	Vitamin E lowers sperm LPO and protects spermatozoa from loss of motility
Zheng and Zhang (1997)[96]	Motility LPO	2 and 3 h incubation (fertile and infertile)	Ferulic acid improves sperm motility and reduces LPO Ferulic acid increases sperm cAMP and cGMP
Calamera *et al.* (2001)[97]	Motility ROS	2–47 h incubation	Catalase did not protect spermatozoa from loss of motility
Chi *et al.* (2008)[98]	Motility ROS	Centrifugation (1000 rpm min^{-1} ×2)+1 h incubation	EDTA or catalase lower CF-induced sperm ROS EDTA (but not catalase) protects spermatozoa from CF-induced loss sperm motility
Donnelly *et al.* (2000)[99]	Motility	Percoll DGC+4 h incubation	GSH or hypotaurine do not protect spermatozoa from loss of motility
DNA integrity			
Chi *et al.* (2008)[98]	COMET	Centrifugation (1000 rpm min^{-1} ×2)+1 h incubation	EDTA or catalase lower centrifugation-induced sperm ROS EDTA or catalase lower centrifugation-induced sperm DD EDTA or catalase have no protective effect on LPO
Donnelly *et al.* (2000)[99]	COMET	Percoll DGC±H_2O_2	GSH, hypotaurine or both do not alter baseline sperm DD GSH, hypotaurine or both do not alter sperm motility at 4 h GSH and/or hypotaurine lower H_2O_2-induced sperm DD
Donnelly *et al.* (1999)[100]	COMET	Percoll DGC	Vitamin C or E do not lower baseline sperm ROS and DD Vitamin C or E protect sperm from H_2O_2 induced ROS and DD Vitamins C+E induce sperm DD and increase H_2O_2-induced DD
Hughes *et al.* (1998)[101]	COMET	Percoll DGC	Vitamins C, E or urate lower sperm DD after DGC Vitamins C+E or AC increase sperm DD after DGC

Abbreviations: AC, acetyl cysteine; CF, centrifugation; COMET, alkaline single-cell gel electrophoresis; DD, DNA damage; DGC, density-gradient centrifugation; DTT, dithiotreitol; GSH, glutathione; LPO, lipid peroxidation; NAC, *N*-acetyl-L-cysteine; ROS, reactive oxygen species; SOD, superoxide dismutase.

Table 6 The role of *in vitro* antioxidants in protecting human sperm DNA from injury caused by cryopreservation and thawing

Study	*Assay*	*Antioxidant*	*Effect of antioxidant on cryopreservation and thawing*
Branco *et al.* (2009)[109]	COMET	Resveratol or ascorbic acid	Improved sperm DNA integrity
Li *et al.* (2009)[110]	COMET	Catalase or ascorbic acid	Improved sperm DNA integrity Reduced ROS production
Martinez-Soto *et al.* (2009)[111]	TUNEL	Genistein	Improved sperm DNA integrity Reduced ROS production Improved post-thaw motility
Thompson *et al.* (2009)[112]	8-OHdG TUNEL	Genistein	Improved sperm DNA integrity (reduced oxidative damage)
Taylor *et al.* (2009)[113]	TUNEL	Vitamin E	No effect on sperm DNA integrity Improved post-thaw motility

Abbreviations: 8-OHdG, 8-hydroxy-2′-deoxyguanosine; COMET, alkaline single cell gel electrophoresis; ROS, reactive oxygen species; TUNEL, terminal nucleotidyl transferase-mediated dUTP nick end labeling.

type of cryoprotectant are also important in improving post-thaw sperm function.[114]

SUMMARY

Oxidative stress plays an important role in the pathophysiology of male infertility. The published studies on dietary antioxidants (including randomized, placebo-controlled trials) generally demonstrate a beneficial effect of antioxidants on sperm function. However, the mechanism of action of these antioxidants as well as the optimal type and dosage of antioxidant is unknown. The study of *in vitro* antioxidants is highly relevant in the era of assisted reproduction because of the susceptibility of human spermatozoa to oxidative injury and the vulnerability of these cells during semen processing. Most studies have demonstrated a beneficial effect of *in vitro* antioxidant supplements in protecting spermatozoa from exogenous oxidants and cryopreservation (with subsequent thawing). In contrast, the effect of these antioxidants in protecting normal spermatozoa from endogenous ROS and gentle sperm processing has not been established conclusively. Additional studies are needed to determine the optimal antioxidant preparation to protect spermatozoa from oxidative stress *in vitro*.

COMPETING FINANCIAL INTERESTS

Dr Armand Zini is a shareholder in YAD technologies Inc. (a nutraceutical supplement company).

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